AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph no. 32 with the following amended paragraph:

In a ninth embodiment, the invention provides methods for the treatment of a subject having a cancer by administering an antibody, antibody fragment or antibody conjugate of the present invention, either alone or in combination with other cytotoxic or therapeutic agents. In particular, preferred cytotoxic and therapeutic agents include docetaxel, paclitaxel, doxorubicin, epirubicin, cyclophosphamide, trastuzumab (Herceptin), capecitabine, tamoxifen, toremifene, letrozole, anastrozole, fulvestrant, exemestane, goserelin, oxaliplatin, carboplatin, cisplatin, dexamethasone, antide, bevacizumab (Avastin), 5-fluorouracil, leucovorin, levamisole, irinotecan, etoposide, topotecan, gemcitabine, vinorelbine, estramustine, mitoxantrone, abarelix, zoledronate, streptozocin, rituximab (Rituxan), idarubicin, busulfan, chlorambucil, fludarabine, imatinib, cytarabine, ibritumomab (Zevalin), tositumomab (Bexxar™), interferon alpha-2b, melphalam, bortezomib (Velcade), altretamine, asparaginase, gefitinib (Iressa), erlonitib (Tarceva), anti-EGF receptor antibody (Cetuximab, Abx-EGF), and an epothilone. More preferably, the therapeutic agent is a platinum agent (such as carboplatin, oxaliplatin, cisplatin), a taxane (such as paclitaxel, docetaxel), gemcitabine, or camptothecin.

Please add the following paragraphs after the last sentence of paragraph no. 92:

"Remission-Induction Chemotherapy

ALKYLATING AGENTS WITH OR WITHOUT GLUCOCORTICOIDS.

A variety of simple alkylating agent-steroid combinations, as well as more complex regimens, have been used for remission induction for patients with multiple myeloma. Overall objective response rates in various series using single alkylating agents alone or in combination with prednisone usually are 50% to 70%, and the rates are influenced by the response criteria used and the aggressiveness with which the regimens can be administered because of their myelosuppressive effects. Prednisone and other glucocorticoids (e.g., methylprednisolone, dexamethasone) have been combined with alkyl-acing agents because of their single-agent

activity, lack of overlapping toxicity, and the suggestion that they may potentiate the action of other agents. In most instances, patients in these trials received maintenance chemotherapy after remission induction.

TABLE 45.4-8. Conventional Intermittent Schedules of Alkylating Agents for Treatment of Myeloma Alone or in Combination With Prednisone

Route	Dose and Schedule
CYCLOPHOSPHAMIDE	
IV	1000 mb/m^2 (27 mg/kg) q 3 wk
Oral	250 mg/m^2/d x 4 d q 3 wk
MELPHALAN	
IV	16 mg/m^2 q 2 wk x 4 then q 4 wk Reduce initial dosing by 50% if serum creatinine > 2 mg/dL (BUN > 30 mg/dL)
Oral	8 mg/m^2 q 3 wk or 9 mg/m2 q 4 wk (Because of varying bioavailability of oral melphalan, the dose must be increased to induce hematologic toxicity or significant underdoseing may occur.)
CARMUSTINE (BCNU)	
IV	100-150 mg/m^2 q 4-6 wk
LOMUSTINE (CCNU)	
Oral	130 mg/m^2 q 4-6 wk
BUN, blood urea nitrogen.	

MULTIAGENT COMBINATION CHEMOTHERAPY.

A number of studies have compared the simple oral melphalan plus prednisone (MP) or cyclophosphamide plus prednisone (CP) combinations with more complex multiagent regimens. Although a subset of these studies reports significantly better survival results than have been observed with the simple combinations, differences are generally minimal at best. Such studies were initiated because of preclinical evidence suggesting that combinations of alkylating agents

may be potentiating because there are different mechanisms of membrane uptake and other potential differences in their mode of action and cellular cytotoxicity.

Some of the most widely used multiagent combinations include the M2 protocol developed at Memorial Sloan-Kettering Cancer Center and the alternating combination chemotherapy regimens developed by SWOG. In the initial SWOG report of alternating combinations, vincristine, melphalan, carmustine, and prednisone (VMCP) was alternated with vincristine, carmustine, doxorubicin, and prednisone (VBAP) or vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP). In subsequent trials, the alternation has been limited to VMCP and VBAP, because VBAP can reinduce remission in myeloma patients who have previously responded and relapsed from therapy with melphalan or cyclophosphamide ... combinations. The dosage schedules for these Memorial Sloan-Kettering and SWOG combination programs are summarized in Table 45.4-10. The fifth Medical Research Council (MRC) trial of alternating combination chemotherapy used drug dosages that were essentially identical with that of SWOG, with the deletion of vincristine and prednisone (see Table 45.4-10). In a recent SWOG study, the VMCP/VBAP regimen was compared with this same program as well as with the addition of alternate-day prednisone (50 mg) between chemotherapy courses (VMCPP/VBAPP) and to the vincristine, doxorubicin, dexamethasone (VAD) regimen.

STUDIES OF INTERFERON-α ALONE OR IN COMBINATION THERAPY FOR REMISSION INDUCTION

Although INF-α is known to have some activity in myeloma patients in relapse, the recombinant forms of IFN-α have had only limited study in previously untreated patients. In an initial report, 7 of 14 patients with previously untreated myeloma with stages I or II myeloma responded to treatment. The response was ... associated with an increase in residual polyclonal immunoglobulins. However, two randomized trials comparing initial therapy with IFN-α to chemotherapy have shown IFN-α monotherapy to be less active than standard chemotherapy. Recombinant IFN-α has also been integrated into combination chemotherapy with alkylating agent and prednisone combinations. On the basis of the initial experience with this approach, the CALGB initiated a randomized trial comparing the effectiveness of MP to MP plus recombinant

IFN-α. This study, as well as that by the Myeloma Group of Central Sweden, failed to show overall benefit from the addition of IFN-α to melphalan-prednisone. Several more recent trials have also failed to show significant overall survival benefit in myeloma by adding IFN to commonly used multiagent induction chemotherapy regimens. A trial conducted by ECOG evaluated the addition of IFN- α2 or high-dose cyclophosphamide to the VBMCP regimen as. compared with VBMCP. Although this large, randomized trial reported a higher "complete remission" rate with the IFN-containing combination compared With VBMCP alone (17% versus 10%), there was no difference in overall response or survival compared with the other induction regimens tested. An additional recent randomized trial conducted in France using the VMCP/VBAP regimen with or without IFN-α also failed to show any benefit from the addition of IFN.

HIGH—DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL RESCUE.

For more than a decade, it has been clear that use of high-dose chemotherapy (*e.g.*, with intravenous melphalan at 2 to 3 times the normal dosage range) either used alone or with autologous hematopoietic stem cell rescue could improve the apparent "complete remission" rate for patients with multiple myeloma in relapse. Such results have been obtained at the cost of the substantial toxicity associated with severe bone marrow aplasia, which routinely occurs after high-dose chemotherapy. A number of single institutions and groups since conducted studies of high-dose chemotherapy with melphalan or other agents along with the use of hematopoietic growth factors and autologous hematopoietic stem cell rescue with peripheral blood stem cells (PBSC) or bone marrow stem cells (BMSC) or the combination for previously untreated patients with myeloma. Use of hematopoietic growth factors plus stem cells in general permits higher doses to be administered than with high-dose chemotherapy alone, and shortens the time required for recovery of bone marrow function after chemotherapy-induced marrow aplasia. These programs were initiated after it was determined that, in refractory patients, high-dose chemotherapy with autologous stem cell rescue could be initiated with a relatively low mortality rate associated with the procedure.

Remission Maintenance Versus Unmaintained Remission

An approach to remission maintenance that used recombinant IFN-α was reported by the Italian Multiple Myeloma Study Group. In this study, 70 patients with remissions induced with MP or VMCP–VBAP (on a randomized induction) were rerandomized to maintenance therapy with recombinant IFN-α2 or to no treatment. The IFN-α2 was administered at a dosage of 3 X 10^6 IU/m^2 subcutaneously three times weekly. After 27 months of follow-up, 8 (24%) of 33 of evaluable patients receiving IFN-α2 and 22 (59%) of 37 patients with no maintenance had relapsed, with a significant difference ($P < .01$) in the actuarial curves of remission duration in the two groups. A larger study of IFN maintenance conducted by the SWOG with approximately 200 patients randomized to IFN maintenance or observation using 3 X 10^6 of IFN-α given intravenously with the same schedule showed no advantage of IFN-α2 over unmaintained remission for remission duration or survival (Fig. 45.4-10). A number of additional IFN-α studies for remission maintenance have subsequently been performed and none of these large-scale studies has shown an improvement in overall survival, although several suggest an increase in the time from start of maintenance to relapse.

BISPHOSPHONATES

New-generation bisphosphonates may actually prevent the development of bony metastases. In several animal models, injected tumor cells failed to establish colonies in bone that had received pretreatment by bisphosphonate. Conceivably, this could translate into the clinical situation. For tumors with certain histologic or molecular features, bisphosphonates could even be considered while treating the primary tumor. A more conventional mode is to use the bisphosphonates to treat hypercalcemia, stop bone reabsorption, and reduce pain from established bone metastases.

Clinical Trials With Erythropoietin

Use of EPO in anemia associated with cancer has been extensively investigated. Trials have been conducted in patients with anemia due to marrow involvement with lymphoproliferative disorders; myelodysplastic syndromes, or solid tumors; and in patients

developing anemia after chemotherapy, autologous transplantation, allogeneic transplantation, or irradiation therapy, and in patients with anemia of cancer. Responses, generally defined as an increase in hemoglobin or a decrease in transfusion requirements, are quite high in most groups of patients, typically in the area of 50%. Lack of response correlates with high pretreatment serum level of endogenous erythropoietin. Response has been associated with improved performance status and quality of life.

TABLE 54.1-15. Adjuvant Analgesics in the Management of Cancer Pain

ADJUVANT DRUGS FOR NEUROPATHIC PAIN
Antidepressants
Anticonvulsants
Oral and cutaneous local anesthetics
Corticosteroids
Clonidine
Benzodiazepines
Neuroleptics
α2-Adrenergic agonists
NMDA antagonists
Calcitonin
ADJUVANT DRUGS FOR BONE PAIN
Bisphosphonates
Gallium nitrate
Calcitonin
Strontium-S9
ADJUVANTS TO TREAT SIDE EFFECTS
Antiemetics
Compazine
Metaclopromide
Ondansteron
Psychostimulants
Caffeine
Methylphenidate
Dextroamphetamine
Laxatives
Senna
ADJUVANTS TO ENHANCE ANALGESIA
Acetaminophen
NSAIDs
Hydroxyzine

ADJUVANTS FOR BONE PAIN.

Metastatic disease to bone is the most common cause of pain in patients with cancer. Analgesic drug therapy is commonly used to manage the pain during the initial treatment with either chemotherapy or radiation therapy. Multifocal metastatic bone disease that is refractory to routine treatments may benefit from the use of a series of agents, including the bisphosphonate compounds, gallium nitrate, calcitonin, and strontium 89. The current bisphosphonates used for the treatment of bone pain include pamidronate and clonidronate. Pamidronate is ustially administered as a brief infusion in a starting dose of 60 mg. Analgesia, if it occurs, usually appears within days, but may accrue for many weeks with repeated infusions. Clonidronate may be administered orally and has been demonstrated to be efficacious in patients with breast cancer and multiple myeloma. Calcitonin has also been reported anecdotally to be useful in patients with malignant bone pain, but the appropriate dose and dosing frequency have not been well defined.

OTHER ANGIOGENESIS INHIBITORS IN CLINICAL TRIALS

Other angiogenesis inhibitors have entered clinical trials for patients with advanced cancer. These include carboxyaminotriazole, a signal transduction inhibitor which blocks calcium influx; Tecogalen (DS4152), a sulfated polysaccharide; Linomide (quinoline-3-carboxamide); thalidomide; BB2516 (British Biotechnology 2516), a metalloproteinase inhibitor which also inhibits angiogenesis and interleukin-12. It is too early to summarize the results of these trials."

Please replace the paragraph no. 93 with the following amended paragraph:

The therapeutic agents that can be combined with EM164 for improved anti-cancer efficacy include diverse agents used in oncology practice (*Reference*: Cancer, Principles & Practice of Oncology, DeVita, V. T., Hellman, S., Rosenberg, S. A., 6th edition, Lippincott-Raven, Philadelphia, 2001), such as docetaxel, paclitaxel, doxorubicin, epirubicin, cyclophosphamide, trastuzumab (Herceptin), capecitabine, tamoxifen, toremifene, letrozole, anastrozole, fulvestrant, exemestane, goserelin, oxaliplatin, carboplatin, cisplatin,

dexamethasone, antide, bevacizumab (Avastin), 5-fluorouracil, leucovorin, levamisole, irinotecan, etoposide, topotecan, gemcitabine, vinorelbine, estramustine, mitoxantrone, abarelix, zoledronate, streptozocin, rituximab (Rituxan), idarubicin, busulfan, chlorambucil, fludarabine, imatinib, cytarabine, ibritumomab (Zevalin), tositumomab (Bexxar™), interferon alpha-2b, melphalam, bortezomib (Velcade), altretamine, asparaginase, gefitinib (Iressa), erlonitib (Tarceva), anti-EGF receptor antibody (Cetuximab, Abx-EGF), epothilones, and conjugates of cytotoxic drugs and antibodies against cell-surface receptors. Preferred therapeutic agents are platinum agents (such as carboplatin, oxaliplatin, cisplatin), taxanes (such as paclitaxel, docetaxel), gemcitabine, and camptothecin.

Please replace the paragraph no. 101 with the following amended paragraph:

Preferably, the therapeutic agent used in the kit is selected from the group consisting of docetaxel, paclitaxel, doxorubicin, epirubicin, cyclophosphamide, trastuzumab (Herceptin), capecitabine, tamoxifen, toremifene, letrozole, anastrozole, fulvestrant, exemestane, goserelin, oxaliplatin, carboplatin, cisplatin, dexamethasone, antide, bevacizumab (Avastin), 5-fluorouracil, leucovorin, levamisole, irinotecan, etoposide, topotecan, gemcitabine, vinorelbine, estramustine, mitoxantrone, abarelix, zoledronate, streptozocin, rituximab (Rituxan), idarubicin, busulfan, chlorambucil, fludarabine, imatinib, cytarabine, ibritumomab (Zevalin), tositumomab (Bexxar™), interferon alpha-2b, melphalam, bortezomib (Velcade), altretamine, asparaginase, gefitinib (Iressa), erlonitib (Tarceva), anti-EGF receptor antibody (Cetuximab, Abx-EGF), and an epothilone. More preferably, the therapeutic agent is a platinum agent (such as carboplatin, oxaliplatin, cisplatin), a taxane (such as paclitaxel, docetaxel), gemcitabine, or camptothecin.

Please replace the paragraph no. 104 with the following amended paragraph:

Based on the efficacy of EM164 antibody as a single agent in inhibiting the proliferation and survival of diverse human cancer cell lines as shown in Table 1, additional efficacy studies were carried out using combinations of EM164 antibody with other anti-cancer therapeutic agents. In these studies on diverse cancer cell lines, the combined treatment of EM164 antibody

and other anti-cancer therapeutic agents resulted in an even greater anti-cancer efficacy than with either EM164 or the other therapeutic agent alone. These combinations of EM164 with other therapeutic agents are therefore highly effective in inhibiting the proliferation and survival of cancer cells. The therapeutic agents that can be combined with EM164 for improved anti-cancer efficacy include diverse agents used in oncology practice (*Reference*: Cancer, Principles & Practice of Oncology, DeVita, V. T., Hellman, S., Rosenberg, S. A., 6th edition, Lippincott-Raven, Philadelphia, 2001), such as docetaxel, paclitaxel, doxorubicin, epirubicin, cyclophosphamide, trastuzumab (Herceptin), capecitabine, tamoxifen, toremifene, letrozole, anastrozole, fulvestrant, exemestane, goserelin, oxaliplatin, carboplatin, cisplatin, dexamethasone, antide, bevacizumab (Avastin), 5-fluorouracil, leucovorin, levamisole, irinotecan, etoposide, topotecan, gemcitabine, vinorelbine, estramustine, mitoxantrone, abarelix, zoledronate, streptozocin, rituximab (Rituxan), idarubicin, busulfan, chlorambucil, fludarabine, imatinib, cytarabine, ibritumomab (Zevalin), tositumomab (Bexxar™), interferon alpha-2b, melphalam, bortezomib (Velcade), altretamine, asparaginase, gefitinib (Iressa), erlonitib (Tarceva), anti-EGF receptor antibody (Cetuximab, Abx-EGF), epothilones, and conjugates of cytotoxic drugs and antibodies against cell-surface receptors.

Please replace the paragraph no. 162 with the following amended paragraph:

A molecular model of murine EM164 was generated using the Oxford Molecular software package AbM. The antibody framework was built from structure files for the antibodies with the most similar amino acid sequences, which were 2jel for the light chain and 1nqb for the heavy chain. The non-canonical CDRs were built by searching a C-α ~~C-a~~-structure database containing non-redundant solved structures. Residues that lie within 5Å ~~2~~-of a CDR were determined.

Please replace the paragraph no. 163 with the following amended paragraph:

The surface positions of murine EM164 were compared to the corresponding positions in human antibody sequences in the Kabat database (Johnson, G. and Wu, T. T. (2001) Nucleic Acids Research, 29: 205-206). The antibody database management software SR (Searle 1998)

was used to extract and align the antibody surface residues from natural heavy and light chain human antibody pairs. The human antibody surface with the most identical surface residues, with special consideration given to positions that come within 5Å ~~?~~ of a CDR, was chosen to replace the murine anti-IGF-I receptor antibody surface residues.

Please replace the paragraph no. 164 with the following amended paragraph:

PCR mutagenesis was performed on the murine EM164 cDNA clone (above) to build the resurfaced, human EM164 (herein huEM164). Primer sets were designed to make the 8 amino acid changes required for all tested versions of huEM164, and additional primers were designed to alternatively make the two 5Å ~~?~~ residue changes (Table 3). PCR reactions were performed with the following program: 1) 94 °C 1 min, 2) 94 °C 15 sec, 3) 55 °C 1 min, 4) 72 °C 1 min, 5) cycle back to step #2 29 times, 6) finish with a final extension step at 72 °C for 4 min. The PCR products were digested with their corresponding restriction enzymes and were cloned into the pBluescript cloning vectors as described above. Clones were sequenced to confirm the desired amino acid changes.

Please replace the paragraph no. 167 and its heading with the following amended heading and paragraph:

F. Molecular modeling to determine which residues fall within 5 Å ~~?~~ of a CDR

The molecular model above, generated with the AbM software package, was analyzed to determine which EM164 surface residues were within 5 Å ~~?~~ of a CDR. In order to resurface the murine EM164 antibody, all surface residues outside of a CDR should be changed to the human counterpart, but residues within 5 Å ~~?~~ of a CDR are treated with special care because they may also contribute to antigen specificity. Therefore, these latter residues must be identified and carefully considered throughout the humanization process. The CDR definitions used for resurfacing combine the AbM definition for heavy chain CDR2 and Kabat definitions for the remaining 5 CDRs (Figure 14). Table 6 shows the residues that were within 5 Å ~~?~~ of any CDR residue in either the light or heavy chain sequence of the EM164 model.

Please replace the Table 6 with the following amended Table 6:

TABLE 6 EM164 antibody framework surface residues within 5 Å ~~?~~ of a CDR

Please replace the paragraph no. 168 with the following amended paragraph:

Candidate human antibody surfaces for resurfacing EM164 were identified within the Kabat antibody sequence database using SR software, which provided for the searching of only specified residue positions against the antibody database. To preserve the natural pairings, surface residues of both the light and heavy chains were compared together. The most homologous human surfaces from the Kabat database were aligned in rank order of sequence identity. The top 5 surfaces are given in Table 7. These surfaces were then compared to identify which of them would require the least changes within 5 Å ~~?~~ of a CDR. The Leukemic B-cell antibody, CLL 1.69, required the least number of surface residue changes (10 in total) and only two of these residues were within 5Å ~~?~~ of a CDR.

Please replace Table 7 with the following Table 7:

TABLE 7 - The top 5 human sequences extracted from the Kabat database

5 Most Homologous Human Antibody Surfaces		
Antibody	Light Chain	SEQ ID NO:
MuEM164	D L T L L Q P G Q K G D S R E K K R A	33
CLL1.69	D V T L L P P G Q R G D A R E K K R -	34
MSL5	D Q S L I P P G Q K G D S R D K K R A	35
CDP571	D M S S V R P G Q K G S S S D K K R -	36
LC3aPB	E V S G P R P G Q R G D S R E K K R -	37
SSbPB	E V S G P R P G Q R G D S R E K K R -	38
Antibody	Heavy Chain	SEQ ID NO:
MuEM164	Q Q Q A L K P G K K T P G Q E K K R K S S S E A S	39
CLL1.69	Q Q V A V K P G K K T P G Q Q K Q G K S S S E Q S	40
MSL5	Q Q Q P L K P G K K T P G K D D K G T S N N E Q S	41
CDP571	Q Q V A V K P G K K T P G Q Q K K G K S S S E Q S	42
LC3aPB	- Q V A V K P G K K T P G Q Q K Q G K S S S E Q S	43
SSbPB	- Q V A V K P G K K T P G Q Q K Q G E S S S E Q S	44

Alignments were generated by SR (Pedersen 1993). The EM164 surface residues that come within 5 Å of a CDR are underlined.

Please replace the paragraph no. 170 with the following amended paragraph:

The ten surface residue changes for EM164 (Table 7) were made using PCR mutagenesis techniques as described above. Because eight of the surface residues for CLL 1.69 were not within 5Å of a CDR, these residues were changed from murine to human in all versions of humanized EM164 (Tables 8 and 9). The two light chain surface residues that were within 5 Å of a CDR (Kabat positions 3 and 45) were either changed to human or were retained as murine. Together, these options generate the four humanized versions of EM164 that were constructed (Figures 22 and 23).

Please replace the paragraph no. 171 with the following amended paragraph:

Of the four humanized versions, version 1.0 has all 10 human surface residues. The most conservative version with respect to changes in the vicinity of the CDR is version 1.1, which

retained both of the murine surface residues that were within 5Å ~~?~~ of a CDR. All four humanized EM164 antibody genes were cloned into an antibody expression plasmid (Figure 16) for use in transient and stable transfections.

Please replace the paragraph no. 174 with the following amended paragraph:

The plot of ([bound murine Ab]/[bound humanized Ab]) *vs* ([murine Ab]/[humanized Ab]) yielded a straight line ($r^2 = 0.996$) with slope ($= K_{d\ humanized\ Ab} / K_{d\ murine\ Ab}$) of 0.52. The humanized antibody version 1.0 therefore bound to IGF-I receptor more tightly than did murine EM164 antibody. Similar values for the gradient, ranging from about 0.5 to 0.8, were obtained for competitions of versions 1.0, 1.1, 1.2 and 1.3 of humanized EM164 antibodies with murine EM164 antibody for binding to full-length IGF-I receptor or to truncated IGF-I receptor alpha chain, which indicated that all of the humanized versions of EM164 antibody had similar affinities, which were all better than that of the parent murine EM164 antibody. A chimeric version of EM164 antibody with 92F→~~?~~C mutation in heavy chain showed a slope of about 3 in a similar binding competition with murine EM164 antibody, which indicated that the 92F→~~?~~ C mutant of EM 164 had a 3-fold lower affinity than did murine EM164 antibody for binding to IGF-I receptor. The humanized EM164 v1.0 antibody showed a similar inhibition of IGF-I - stimulated growth and survival of MCF-7 cells as did the murine EM164 antibody (Figure 24). The inhibition of serum-stimulated growth and survival of MCF-7 cells by humanized EM164 v1.0 antibody was similar to the inhibition by murine EM164 antibody.